proper intracellular processing and transport to the plasma membrane. Unlike wild type rBAT, the mutant proteins were primarily located in an intracellular compartment, most likely the ER. Evidence also suggested that, if able to reach the cell surface, as is the case if the experimental system is saturated with cDNA encoding the mutant, the mutant proteins are functional. As for the other mutant proteins described herein, it is likely that mutations in rBAT lead to misfolding and retention in the ER. Thus agents, such as those described herein, that increase or stimulate the release of misfolded proteins from the ER may be useful in the prevention or treatment of Type I cystinuria (and possibly other forms of cystinuria that may involve rBAT) by allowing mutant rBAT to exit the ER and reach the cell surface.

With respect to the disorders and conditions discussed above, in certain embodiments of the invention the method for treatment and/or prevention or prophylaxis comprises administering an agent that permits the release of proteins from the endoplasmic reticulum, an agent that decreases or inhibits the activity of UDP glucose:glycoprotein glycosyl transferase, an agent that decreases or inhibits activity of the endoplasmic reticulum Ca^{++} ATPase, an agent that lowers the concentration of Ca^{++} in the endoplasmic reticulum, an agent that causes release of Ca++ from the ER, an agent that decreases or inhibits IP_3 receptor activity, an agent that decreases or inhibits calnexin functional activity, or an agent that increases or activates ryanodine receptor activity. Particular agents that may be used in the practice of the invention include thapsigargin or a derivative thereof, cyclopiazonic acid or a derivative thereof, DBHQ or a derivative thereof, and halothane or a derivative thereof.

K. Thapsigargin

General Description. Thapsigargin and related sesquiterpene lactones are naturally-occurring compounds known to selectively inhibit all of the SERCA ATPases, a family of Ca^{+2}-pumping ATPases present in the ER of all mammalian cells, with subnanomolar potency. These inhibitors have no effect on the Ca^{+2}-ATPase of the plasma membrane or on other P-type ATPases. Members of this class of inhibitors include thapsigargin and thapsigargicin, both isolated from *Thapsia garganica*, thapsivillosin A (TvA), isolated from *Thapsia villosa*, and trilobolide, extracted from *Laser trilobum* (Wictome *et al.*, Biochem. J. 310:859-868 (1995)).

Functional Role. Thapsigargin appears to induce a conformational state of the pump in which several of the partial reactions (*e.g.*, Ca^{+2} binding, Ca^{+2}-independent phosphorylation by P_i, nucleotide binding) are blocked (Inesi *et al.*, Arch. Biochem.

Biophys. 298:313-317 (1992)). Studies utilizing a series of thapsigargin analogues indicated that the compound fits into a sterically discriminating cleft involving the hydrophobic transmembrane region of the ATPases (Christensen *et al.*, Federation of European Biochemical Societies 335(3):345-348 (1993)).

Clark *et al.* (J. Orthop. Res. 12(5):601-611 (1994)) reported that "the calcium-mobilizing agents thapsigargin and 2,5-di-(tert-butyl)-1,4-benzohydroquinone were shown to markedly elevate the intracellular calcium concentration of chick embryo chondrocytes in a dose-dependent manner." The observed effects of the two compounds on secretion of chondrocyte proteins, including collagen and proteoglycan, was speculated as being due to the specific depletion of the calcium sequestered in the ER.

Addition of 2 mmol/liter Ca^{+2} to thapsigargin-treated CFPAC-1 cells produced a sustained increase of Cl^- and K^+ currents, which was reversed by Ca^{+2} removal (Galietta *et al.*, Pflugers Arch. 426(6):534-541 (1994)). The researchers concluded "that CFPAC-1 cells respond to nucleotide receptor activation with a transient increase in intracellular Ca^{+2} concentration that stimulates Ca^{+2}-dependent Cl^- and K^+ currents."

It should be noted that it would not be obvious that long term exposure to thapsigargin will increase functional expression of CFTR. For example, down-regulation of CFTR gene expression was observed by others after exposure of HT-29 human colon carcinoma cells to: (1) agents which increase intracellular divalent cation concentrations (*e.g.*, agents such as the divalent cation ionophores A23187 and ionomycin); (2) thapsigargin; and, (3) growth media containing increased extracellular concentrations of Ca^{+2} or Mg^{+2} (Bargon *et al.*, Mol. Cell. Biol. 12(4):1872-1878 (1992)). These researchers stated that thapsigargin was "an agent that releases Ca^{+2} from intracellular stores" resulting in a higher intracellular level of divalent cation concentration. The authors concluded that "despite the independence of Ca^{+2}-dependent Cl^- channels and cyclic AMP-dependent CFTR-related Cl^- channels in epithelial cells, increases in intracellular divalent cation concentrations down-regulate the expression of the CFTR gene at the transcriptional level, with consequent decreases in CFTR mRNA and protein."

Exposure of tumor sections from BALB/Urd mice to ionomycin or thapsigargin resulted in a concomitant efflux of ^{125}I, ^{36}Cl and ^{86}Rb (Basavappa *et al.*, *Gastroenterology* 104(6):1796-1805 (1993)).

L. Recombinant DNA

In accordance with the present invention, as described above or as discussed in the Examples below, there may be employed conventional molecular biology, microbiology

and recombinant DNA techniques. Such techniques are explained fully in the literature. See for example, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (Second Ed., Cold Spring Harbor Press, Cold Spring Harbor NY, 1989); DNA Cloning: A Practical Approach, vol. 1 and 2 (D.N. Glover ed., 1985); Oligonucleotide Synthesis (M.J. Gait ed., 1984); Nucleic Acid Hybridization (B.D. Hames *et al.*, 1985); Transcription and Translation (B.D. Hames *et al.*, eds, 1984); E. Harlow *et al.*, Antibodies: A Laboratory Manual (Cold Spring Harbor Press, Cold Spring Harbor NY, 1988); Roe *et al.*, DNA Isolation and Sequencing: Essential Techniques (John Wiley & Sons, NY, 1996) and Ausubel *et. al.*, Current Protocols in Molecular Biology (Greene Publishing Co. NY, 1995) to name a few.

For recombinant procedures related to treating cystic fibrosis see, for example, U.S. Patent Nos. 5,602,110, 5,674,898 and 5,707,855.

M. Antisense RNA

Antisense molecules are RNA or single-stranded DNA molecules with nucleotide sequences complementary to a specified mRNA. When a laboratory-prepared antisense molecule is injected into cells containing the normal mRNA transcribed by a gene under study, the antisense molecule can base-pair with the mRNA, preventing translation of the mRNA into protein. The resulting double-stranded RNA or RNA/DNA is digested by enzymes that specifically attach to such molecules. Therefore, a depletion of the mRNA occurs, blocking the translation of the gene product so that antisense molecules find uses in medicine to block the production of deleterious proteins. Methods of producing and utilizing antisense RNA are well known to those of ordinary skill in the art (see, for example, C. Lichtenstein and W. Nellen (Editors), Antisense Technology: A Practical Approach, Oxford University Press (December, 1997); S. Agrawal and S.T. Crooke, Antisense Research and Application (Handbook of Experimental Pharmacology, Volume 131), Springer Verlag (April, 1998); I. Gibson, Antisense and Ribozyme Methodology: Laboratory Companion, Chapman & Hall (June, 1997); J.N.M. Mol and A.R. Van Der Krol, Antisense Nucleic Acids and Proteins, Marcel Dekker; B. Weiss, Antisense Oligodeoxynucleotides and Antisense RNA: Novel Pharmacological and Therapeutic Agents, CRC Press (June, 1997); Stanley *et al.*, Antisense Research and Applications, CRC Press (June, 1993); C. A. Stein and A. M. Krieg, Applied Antisense Oligonucleotide Technology (April, 1998)).

Antisense molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include